Mycostimulator of Chitinolytic Activity: Thermodynamic Studies and its Activity against Human and Food-Borne Microbial Pathogens

Marwah M. Bakri,[a] Aisha M. H. Al-Rajhi,[b] Emad Abada,[a] Olfat M.A. Salem,[c] Abdel-Rahman M. Shater,[a,d] Mohamed S. Mahmoud,[e] and Tarek M. Abdel Ghany [f,*]

Chitinolytic activity and antibiosis are gaining prominence in various biotechnological fields. Dead fungal biomass (DFB) was used as a mycostimulator of chitinase production and antibiosis by *Aspergillus fumigatus*. The presence of DFB stimulated the synthesis of various secondary metabolites by *A. fumigatus* that were detected by gas chromatography-mass spectrometry analysis such as 6,8-Di-C-á-glucosylluteolin; bistrimethylsilyl N-acetyl eicosasphinga-4,11-dienine; curan-17-oic acid, 19,20-dihydroxy-, methyl ester, (19S)-; spiro[5à-androstane-3,2'-thiazolidine; retinal; Androsta-1,4-dien-3-one; Panaxydol; Costunolide; Cyclo-(glycyl-L-tyrosyl); and 2-amino ethane thiolsulfuric acid. Chitinase activity was 42.9 Units/mL with the presence DFB, where it was 10.3 Units/mL without DFB. The maximum activity of chitinase was observed at 1.5 g of dead fungal biomass, at 4 h, 50 °C and pH 6. Thermodynamic properties showed $\Delta H°$ and $\Delta S°$ values of 126 KJ mol^{-1} and 432 J mol^{-1} K^{-1}, respectively, indicating an endothermic reaction up to 60 °C. Deviation in $\Delta G°$ values confirmed that the reaction at 10 to 20 °C is a nonspontaneous reaction, and at 30 to 60 °C the reaction has a spontaneous nature. DFB encouraged the antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Aspergillus fumigatus*, *Mucor circinelloides*, and *Candida albicans* with 2.3, 2.2, 2.8, 0.8, 0.7, and 2.2 mm inhibition zones, respectively.

DOI: 10.15376/biores.17.3. 4378-4394

Keywords: Mycostimulator; Chitinase; Antibiosis; Fungi; Thermodynamic

*Contact information: a: Biology Department, Faculty of Science, Jazan University, Jazan 82817, Saudi Arabia; b: Department of Biology, College of Science, Princess Nourah bint Abdulrahman University P.O. Box 84428, Riyadh 11671, Saudi Arabia; c: Biology Department, Aldarb College, Jazan University, Jazan d: Biology Department, Faculty of Science, Thamar University, Thamar, Yemen; e: Sanitary and Environmental Engineering Institute (SEI), Housing and Building National Research Center (HBRC), 1770, Giza, Egypt; f: Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo 11725, Egypt; *Corresponding author: tabdelghany.201@azhar.edu.eg*

INTRODUCTION

The cell wall of fungi consists of many components, the most common of which are cellulose and chitin. The latter is considered the main structural component of most fungal mycelia. The composition of chitins were characterized previously as polymers of β-(1,4)-linked-N-acetylglucosamine that are present in un-branched chains in the two large groups of Basidiomycota and Ascomycota (Elieh-Ali-Komi and Hamblin 2016). In nature, chitin represents the next most abundant polysaccharide after cellulose and hemicellulose. In addition to its occurrence in fungi, it is also found in algae, insects, and different

vertebrates (Rana and Farhana 2019). However, most fungi produce many enzymes (Abdelghany and Bakri 2019; Abdelghany *et al.* 2018, 2020), from which chitinase can play an essential role as a defense mechanism against other fungi or to obtain nutrients *via* degradation of substrates containing chitins. Chitinase also has applications in industrial process, agricultural field, food processing (da Silva *et al.* 2005; Nofal *et al.* 2021a,b,c; Al-Rajhi *et al.* 2022a,b). Other applications of chitinases were reported in recent studies. For example, chitinase may be applied in the bioconversion of several wastes resulted from industrial and food process; also it is used in biocomposting of chitin-containing waste and depolymerizing chitosan (Gilvanova *et al.* 2022). Microbial chitinase has been shown to contribute in the biocontrol process against pathogenic fungi including *Fusarium graminearum*, *Magnaporthe oryzae*, *Rhizoctonia solani*, *Botrytis cinerea* and *Puccinia* species (Ekundayo *et al.* 2022). The presence of chitin induces fungal chitinase genes that express to chitinase enzymes (Deng *et al.* 2007). In nature, the generation of some nitrogen and carbon may be due to degradation of chitin *via* chitinase (Rana and Farhana 2019).

The rigidity and construction matrix of the fungal cell wall also depend on glucans as well as chitins. There are two forms of glucans, namely β-glucans, which join through β-(1,3)- or β-(1,6) bonds, and α-glucans, which join through α-(1,3)- and/or α-(1,4) bonds (Webster and Weber 2007). As mentioned previously (El-Tarabily *et al.* 2000), the action of chitinases and glucanase on the microbial cell wall lead to deacylated oligomer chitosan, disaccharide chitobiose, and the monomer N-acetylglucosamine. These changes cause disruption of microbial structure, cellular function, subsequent lysis and death. Biocontrol using microbes depends not only on the mycoparasitic nature but also on the hydrolytic enzymes of the biocontrol agent. Decline in mycelial biomass of pathogens was explained on the basis of lysis of the cell wall *via* lytic enzymes (Chang *et al.* 2007; Dukare *et al.* 2020). Starke *et al.* (2020) studied the attacking dead fungal biomass chitin by *Ewingella americana* pseudomonas isolates and reported the activity of enzyme on the glucans and chitin backbone. Utilization of fungal cell walls as the substrate of chitinase production by *Myrothecium verrucaria* (Vyas and Deshpande 1989) and *Streptomyces viridificans* (Gupta *et al.* 1995) was reported.

From previous indications, antibiosis of microorganisms may depend on the lytic enzymes as well as secretion of antimicrobial compounds; therefore, these enzymes have a significant role as antimicrobial compounds (Bowman and Free 2006; Shaikh and Sayyed 2015). Several secondary metabolites were detected in the fungal cultures and showed antimicrobial activities (Abdelghany 2014). The interaction among fungi, particularly in dual culture, was studied previously to stimulate the yielding of many array of metabolites (Chatterjee *et al.* 2016).

Thermal stability of enzymes is a critical factor of industrial applications of enzymes; therefore, thermodynamic studies have been carried out to support these phenomenon (Karam *et al.* 2017). Different parameters, such as enthalpy (ΔH), Gibbs free energy (ΔG), and entropy (ΔS), were applied to judge the stability and tolerance of enzymes to increased temperature (Mostafa *et al.* 2018). Stimulation of chitinase production and antibiosis by live dual cultures of fungi was reported in many studies, but there has been a lack of reported work concerned with growing live fungus on dead fungal biomass. Therefore, the objective of the current study was achieved *via* dead biomass with thermodynamic studies of chitinase production.

EXPERIMENTAL

Chemicals Used

All used chemicals and growth media contents, including colloidal chitin (crab shell chitin), potato dextrose agar (PDA) medium, Czapek Dox agar medium, malt extract medium, solvents, and buffers, were provided from Sigma-Aldrich, St. Louis, MO, USA.

Selection of Fungi Used in the Experiment

One fungus was isolated from an Oriental hornet (*Vespa orientalis*) trap growing as saprobe on dead Oriental hornets. The isolate was purified and cultivated on different media including PDA, Czapek Dox agar, and malt extract media for identification based on macro- and microscopic examination. The identification was completed according to previous keys (Raper and Fennell 1973; Domsch *et al.* 1980).

Preparation of Culture Filtrates

Dead fungal biomass of *Aspergillus flavus* was used to induce chitinase production. Five g of fungal fresh weight were washed 5 times with distilled water, then autoclaved at 121 °C for 30 min. The autoclaved biomass was added in Czapek Dox broth medium without sucrose, then inoculated with 5-mm diameter disc of active margins of fungus on a PDA plate. The inoculated glass bottle was then incubated at 30 °C, *via* 0.22-µM bacterial proof filter, and the medium broth (200 mL) was filtrated. The filtrate was used as a sources of crude chitinase and crude protein.

Colloidal Chitin Preparation and Assay of Chitinase Activity

A total of 10 g of chitin flakes were added slowly to 175 mL concentrated HCl and mixed gently for 3 h on a magnetic stirrer. This solution was then filtered to 500 mL of pre-chilled, distilled water with constant mixing and allowed to settle. A dense white precipitate formed that was then centrifuged at 10,000 rpm for 10 min at 4 °C. The precipitate was then washed in cold, distilled water repeatedly until the pH of the wash reached 5.5. The supernatant was discarded, and the colloidal chitin was then kept in a refrigerator for future use. Then, 1% of colloidal chitin was added to buffer solution (pH 5) of acetate, followed by sterilization in the autoclave. The enzyme substrate was used for evaluation of chitinase (Endochitinase) activity, which in the current method depended on the turbidity reduction of colloidal suspension containing chitin. The reaction mixture contained enzyme solution and colloidal chitin (1:1 ratio). One enzyme unit was defined as the quantity of enzyme necessary to reduce the turbidity at 510 nm of a chitin suspension by 5% (Tronsmo and Harman 1993). According to Bradford (1976), the protein level was assessed using bovine serum albumin.

Chitinase Activity at Different Conditions

Chitinase activity was studied using different concentrations of dead fungal biomass. The studied concentrations were 0.5, 1, 1.5, 2, 2.5, and 3 g/200 mL of Czapek Dox broth medium inoculated with chitnase producer fungus incubated at 30 °C for 8 days. Furthermore, the activity of chitinase at optimum biomass was evaluated at different interval times ranging from 1 to 7 h. Additionally, the filtrate containing enzyme was incubated for 1 h at different temperatures ranging from 10 to 70 °C to evaluate the activity of chitinase. For the effect of pH on chitinase activity, the Czapek Dox broth medium amended with the optimum dead fungal biomass was adjusted at different pH values

ranging from 3 up to 9, followed by inoculation with chitnase producer fungus and incubated at optimum temperature for 8 d (Abdelghany *et al.* 2020).

Thermodynamic Parameters

Thermodynamic functions can be calculated from the effect of temperature as follows: ΔG, ΔH, and ΔS. The standard free energy change, which is a measure of the spontaneity of the chemical reaction, is expressed as,

$$\Delta G = -RT \ln K_a \quad (1)$$

where R is the gas constant (8.31432 $J \cdot K^{-1} \cdot mol^{-1}$), T is the absolute temperature (standard state = 298 K), and K_a is the equilibrium constant.

In a solid-liquid system K_a can be replaced by the distribution coefficient (K_d). In such a case ΔG is given as,

$$\Delta G = -RT \ln K_d \quad (2)$$

or:

$$\Delta G = -2.303\, RT \log K_d \quad (3)$$

The function ΔG provides a measure of the degree of complex formation. A higher negative ΔG results in a more complete formation of the complex. The free energy change is related to the changes in the enthalpy and entropy according to the following equation,

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

where ΔH is the enthalpy change (KJ mol^{-1}) , ΔS is the entropy change (J K^{-1} mol^{-1}), and T is the absolute temperature (°C).

Fungal Filtrate Extract Analysis *via* Gas Chromatography-Mass Spectrometry (GC-MS)

The used GC (THERMO Scientific Corp., Dani, Rome, Italy)-MS (ISQ Single Quadrupole Mass Spectrometer) for fungal filtrate extract analysis was under the following conditions : Injection of the extract in the capillary column TR-5MS (30 m × 0.32 mm × 0.25 µm) with different temperature cycles including 60 °C, followed by 240 °C, followed by gradually increase 30 °C/min up to 290 °C and held for 2 min. Helium was used as the carrier gas (One mL/min). An autosampler AS1300 (Thermo Fisher Scientific Inc., Dani, Rome, Italy) interconnected to the GC in the split style was used to inject 1 µL of the extract. Through mass spectra and retention time (RT), the detected compounds were identified and compared with identified compounds from the library mass spectra at the National Institute of Standards and Technology (Abdelghany *et al.* 2021).

Antimicrobial Activity of Fungal Extract

The filtrate extract of fungus grown with and without dead fungal biomass was tested against growth of various microorganisms including *Mucor circinelloides*, *Aspergillus fumigatus*, *Candida albicans, Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus subtilis* using the Well agar diffusion protocol (Abdelghany *et al.* 2021). The medium growth was streaked with test microorganism, then a hole of agar was made by a 6-mm sterile cork borer. The dissolved extract in dimethyl sulfoxide (DMSO) (100 µL) was put into the well, followed by incubation of the plat at suitable temperature (37 °C and 25 °C for 24 h and 72 h for bacteria and fungi, respectively). The clear zone around each

well was measured. Gentamycin as antibiotic and Ketoconazole as antifungal were used as control. The DMSO in the well was also used as another control (Qanash *et al.* 2022). Fold increase (FI, %) of the activity of the combination of extract with antibiotic or antifungal was also evaluated *via* the following Eq. 5):

$$FI = \frac{\text{Activity of extract with antibiotic or antifungal} - \text{Activity of antibiotic or antifungal}}{\text{Activity of antibiotic or antifungal}} \times 100 \quad (5)$$

Statistical Analysis

All experimental results were realized in triplicate. The standard deviation (SD) and variance were calculated using SPSS ver. 22.0 software (version 14, IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

The chitinase producer fungus was isolated from dead Oriental hornets (*Vespa orientalis*) in a collected trap (Fig. 1A). It is known that the Oriental hornet is one of the main important insects that attack apiaries, particularly in Arab countries. The fungus was identified as *A. fumigatus.* As a result of fungus isolation from dead Oriental hornets that contain large content of chitin, this fungus was tested for the production of chitinase, but used dead fungal biomass of other fungus as the growth medium (Fig. 1A and AD).

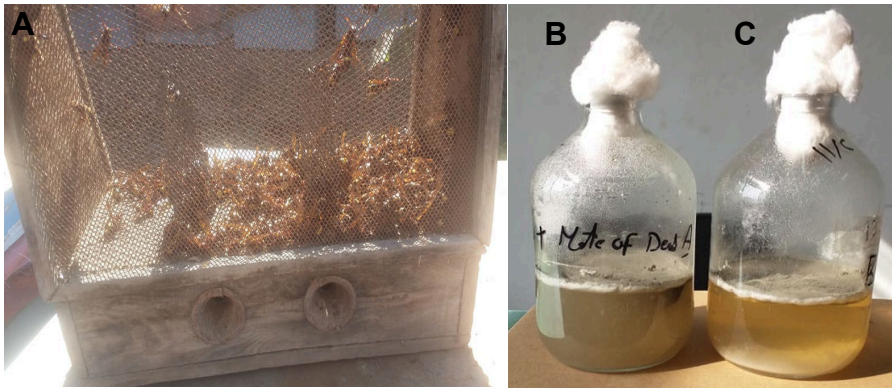

Fig. 1. Oriental hornet trap (A), fungus growing without dead fungal biomass (B), and with dead fungal biomass (C)

The GC-MS analysis of fungal metabolites showed less detected compounds (13 compounds) in the supernatants of fungus culture cultivated without dead fungal biomass (Table 1 and Fig. 2) unlike the number detected of compounds (41 compounds) in the cultivated fungus with dead biomass (Table 2 and Fig. 3), indicating the stimulatory role of dead biomass in the biosynthesis of numerous compounds. Some compounds, including acetic acid ethyl ester, d-gala-l-ido-octonic amide, desulphosinigrin, hexadecanoic acid, and α-D-glucopyranose, 1-thio-, were detected in fungal metabolites cultivated with or without dead biomass, while other compounds were detected only in fungal metabolites cultivated with dead biomass, for example 6,8-di-C-α-glucosylluteolin; bistrimethylsilyl N-acetyl eicosasphinga-4,11-dienine; curan-17-oic acid, 19,20-dihydroxy-,methyl ester, (19S)-; spiro[5α-androstane-3,2'-thiazolidine; retinal; androsta-1,4-dien-3-one; panaxydol; costunolide; cyclo-(glycyl-L-tyrosyl); and 2-amino ethane thiolsulfuric acid**.**

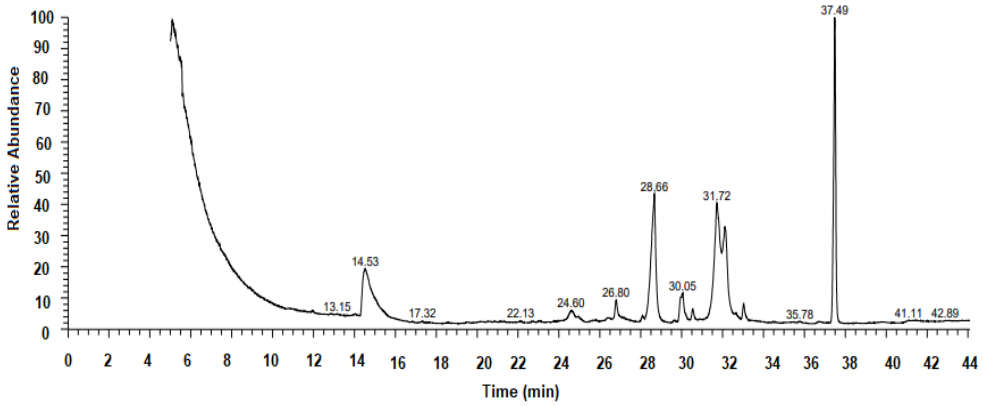

Fig. 2. GC-MS chromatogram analysis of fungus metabolites growing without dead fungal biomass

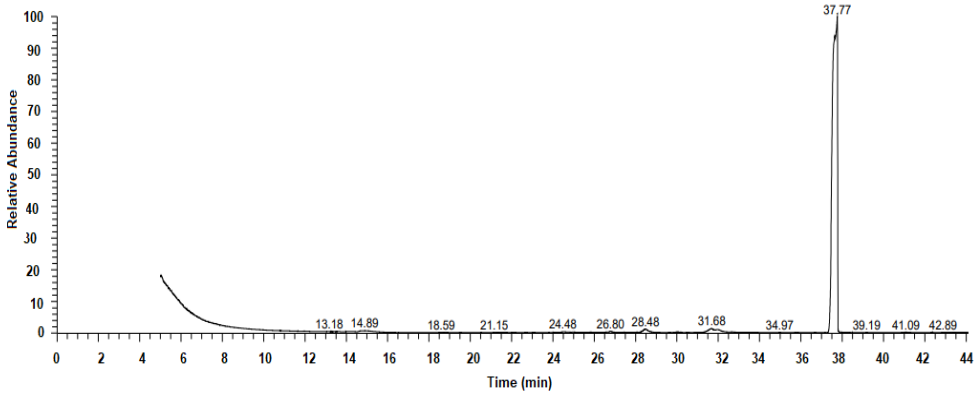

Fig. 3. GC-MS chromatogram analysis of fungus metabolites growing with dead fungal biomass

Table 1. GC-MS Analysis of Fungus Metabolites Growing Without Dead Fungal Biomass

Detected Constituent	RT*	Area (%)	MF*	MW*
Acetic acid ethyl ester	5.36	0.66	$C_4H_8O_2$	88
d-Gala-l-ido-octonic amide	10.92	0.01	C_8H17NO_8	255
Desulphosinigrin	11.04	0.01	$C_{10}H_{17}NO_6S$	279
α-D-glucopyranose, 1-thio-,	11.97	0.02	$C_{10}H_{17}NO_6S$	228
Methyl-9,9,10,10-d4-octadecanoate	26.79	1.95	$C_{19}H_{34}D_4O_2$	302
Hexadecanoic acid	28.66	18.09	$C_{16}H_{32}O_2$	256
9,12-Octadecadienoic acid, methyl ester, (E,E)-	29.95	2.03	$C_{19}H_{34}O_2$	294
11-Octadecenoic acid, methyl ester	30.04	2.82	$C_{19}H_{36}O_2$	296
Methyl 12-(2-octylcyclopropyl) dodecanoate	30.54	1.14	$C_{24}H_{46}O_2$	366
9-Octadecenoic acid (Z)-	31.72	9.21	$C_{18}H_{34}O_2$	282
Octadecanoic acid	32.15	7.42	$C_{18}H_{36}O_2$	284
Tributyl acetylcitrate	33.03	1.60	$C_{20}H_{34}O_8$	402
Diisooctyl phthalate	37.49	33.00	$C_{24}H_{38}O_4$	390
*Note: Retention Time (RT); Molecular formula (MF);Molecular weight (MW)				

Table 2. GC-MS Analysis of Fungus Metabolites Growing with Dead Fungal Biomass

Detected Constituent	RT*	Area (%)	MF*	MW*
Acetic acid ethyl ester	5.61	0.01	$C_4H_8O_2$	88
α-D-glucopyranose, 1-thio-,	10.92	0.01	$C_{10}H_{17}NO_6S$	279
d-Gala-l-ido-octonic amide	11.04	0.01	$C_8H_{17}NO_8$	255
2,3,4,5-Tetrahydroxy pentanal	11.97	0.02	$C_5H_{10}O_5$	150
Desulphosinigrin	13.18	0.01	$C_{10}H_{17}NO_6S$	279
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-Y L) methyl ester, CIS	13.62	0.01	$C_{28}H_{44}O_4$	444
2-Aminoethanethiol hydrogen sulfate (Ester)	14.10	0.03	$C_2H_7NO_3S_2$	175
N-(4,6-Dimethyl-2-pyrimidinyl)-4-(4-nitrobenzylideneamino)benzenesulfonamide	14.46	14.46	$C_{19}H_{17}N_5O_4S$	411
N'-(2,4,6(1H,3H,5H)-Trioxopyrimid in-5-ylidenemethyl)-2-nitrobenzhyd razide	14.79	0.2	$C_{12}H_9N_5O_6$	319
Phenol, 4-(dimethylamino)-3-methy L-, methylcarbamate (Ester)	14.89	0.19	$C_{11}H_{16}N_2O_2$	308
2-Amino ethane thiolsulfuric acid	17.33	0.01	$C_2H_7NO_3S_2$	157
Cyclo-(glycyl-L-tyrosyl)	17.53	0.01	$C_{11}H_{12}N_2O_3$	220
2-Amino-N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]acetamide	18.82	0.01	$C_{11}H_{19}N_3O$	209
Costunolide	19.51	0.02	$C_{15}H_{20}O_2$	232
Panaxydol	20.26	0.01	$C_{17}H_{24}O_2$	260
Androsta-1,4-dien-3-one,	20.53	0.01	$C_{20}H_{28}O_2$	300
N'-(3-Nitro-4-Morpholinobenzylidene)-2-(4-methylphenoxy)acethydrazide	20.86	0.01	$C_{20}H_{22}N_4O_5$	398
1-Methyl N-L-ALPHA-aspartyl-L-phenylalanate	21.15	0.02	$C_{14}H_{18}N_2O_5$	294
N-[4-(3-Hydroxy-1-pyrrolidinyl)-2-butynyl]-N-methylacetamide	21.55	0.01	$C_{11}H_{18}N_2O_2$	210
Formamide, N-methyl-N-4-[1-(pyrrolidinyl)-2-b utynyl]-	21.64	0.01	$C_{10}H_{16}N_2O$	180
Aminoacetamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]-	22.04	0.01	$C_{11}H_{19}N_3O$	209
2-Acetyl-3-(2-cinnamido)ethyl-7-methoxyindole	22.15	0.01	$C_{22}H_{22}N_2O3$	362
2,5-Octadecadiynoic acid, methyl ester	22.69	0.02	$C_{19}H_{30}O_2$	290
Retinal	23.80	0.01	$C_{20}H_{28}O$	284
9,12,15-Octadecatrienoic acid	24.48	0.07	$C_{27}H_{52}O_4Si_2$	496
2,3-Dihydroxypropyl palmitate	24.96	0.02	$C_{19}H_{38}O_4$	330
1-Methyl N-L-ALPHA-aspartyl-L-phenylalanate	25.12	0.01	$C_{15}H_{18}O_3$	246
7-Hydroxy-6,9a-dimethyl-3 -methylene-decahydro-A zuleno[4,5-B]furan-2,9-dione	25.82	0.01	$C_{15}H_{20}O_4$	264
Spiro[5à-androstane-3,2'-thiazolidine	26.38	0.01	$C_{21}H_{35}NS$	333
Cyclopentanetridecanoi c acid, methyl ester	26.80	0.09	$C_{19}H_{36}O_2$	296
Octadecanoic acid, ethyl ester	28.08	0.02	$C_{20}H_{40}O_2$	312
n-Hexadecanoic acid	28.48	0.35	$C_{16}H_{32}O_2$	256
Curan-17-oic acid, 19,20-dihydroxy-, methyl ester	29.64	0.01	$C_{20}H_{26}N_2O_4$,	358
3',4',7-Trimethylquercetin	30.18	0.02	$C_{18}H_{16}O_7$	344
Pentadecanoic acid	30.53	0.03	$C_{17}H_{34}O_2$	270
2-Methylenecholestan-3-OL	34.56	0.01	$C_{28}H_{48}O$	400
Bistrimethylsilyl N-acetyl eicosasphinga-4,11-dienine	34.97	0.01	$C_{28}H_{57}NO_3Si_2$	511
1,1',2,2'-Tetrahydro-PSI,PSI-carotene	35.76	0.02	$C_{42}H_{64}O_2$	600
17,21-Dihydroxypregn-4-ene-3,20-dione bis (O-methyloxime)	36.69	36.69	$C_{23}H_{36}N_2O_4$	404
6,8-Di-C-á-glucosylluteolin	37.94	0.01	$C_{28}H_{25}NO_7$	487
Glycine, N-[(3à,5á,7à,12à)-24-OXO-3,7,12-T RIS[(trimethylsilyl)oxy]C holan-24-YI]-, methyl ester	41.09	0.07	$C_{36}H_{69}NO_6Si_3$	695
*Note: Retention Time (RT); Molecular formula (MF); Molecular weight (MW)				

Brakhage and Schroeckh (2011) mentioned that the creation of new compounds can be stimulated by the co-culturing of microorganisms with other microbes. In another study (Rateb *et al.* 2013), synthesis of various metabolites by *A. fumigatus* was induced by the presence of dead cells of *Streptomyces bullii* in culture medium. Some of the detected compounds reflected antimicrobial activities. For example, *Proteus mirabilis* was inhibited by desulphosinigrin (Vinothkanna *et al.* 2014). However, the retinal and panaxydol were reported as secondary metabolites of some fungi (Prado-Cabrero *et al.* 2007; Schumacher *et al.* 2008; Collado and Viaud 2016), but it was recorded only when the fungus were grown in the presence of dead biomass (Table 2). Panaxydol and 3',4',7-trimethylquercetin displayed antifungal potential towards mucormycosis (Bharat *et al.* 2021). Antimicrobial activity was exhibited using costunolide against *Staphylococcus aureus*, *Mycobacterium tuberculosis, Scopulariopsis* sp., *Candida albicans*, *Aspergillus niger*, *Curvulari lunata*, and *Magnaporthe grisea* (Fischer *et al.* 1998; Luna-Herrera *et al.* 2007; Duraipandiyan *et al.* 2012). Many current detected metabolites were recognized as fungal metabolites in other studies with multiple biological functions. For example, curan-17-oic acid, 19,20-dihydroxy-,methyl ester,(19S)- was detected as one of the metabolites of the current tested fungus (Table 2) and of metabolites of *Candida albicans* in another study (Mohanad *et al.* 2016) and showed antifungal against *Aspergillus fumigatus* and antibacterial activity against *Proteus mirabilis*. Additionally, 7-hydroxy-6,9a-dimethyl-3-methylene-decahydro-A zuleno[4,5-B]furan-2,9-dione was identified as a metabolite of *Pleurotus cornucopiae* and appeared to exhibit antimicrobial activity (Renu and Dinesh 2015).

The isolate fungus was tested for chitinase activity using fungal dead biomass (Fig. 4). The obtained results indicated that the dead biomass stimulated the fungus to produce chitinase (42.9 Units/mL) compared with its productivity (10.3 Units/mL) without dead biomass. The presence of chitin in the cell wall of dead fungal mycelia was the main inducer for chitinase production.

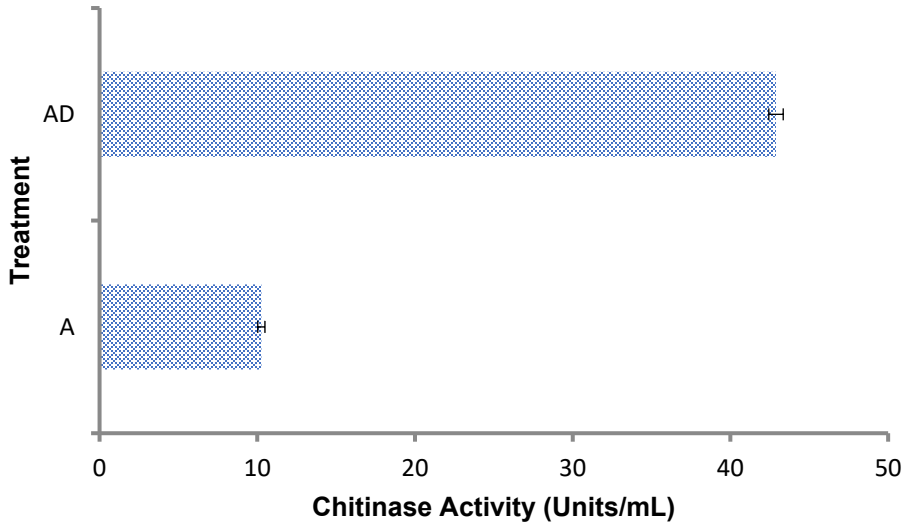

Fig. 4. Chitinase activity of fungus growing without (A) and with dead fungal biomass (AD). Error bars ± indicated standard deviation

Dead fungal biomass was described previously by Baldrian *et al.* (2013), who observed that the polysaccharides represent the main structure (80 to 90%) of the fungal cell wall. From polysaccharides, chitin was detected in a high content of fungal cell walls (Zeglin and Myrold 2013). Ueno *et al.* (1990) mentioned fungal chitinase was able to lyse the dead and live walls of fungi. Different dead fungal biomasses, including *C. lunata*, *A. flavus*, *A. niger*, *F. udum*, and *F. oxysporum*, were used as substrates of chitinase production by *Rhizobium* sp. instead of chitin (Sridevi and Mallaiah 2008), with different activities of enzyme depending on the type of fungal mycelia. The highest activity of chitinase was observed in soils enriched with biomass of fungi subsequently with rapid decomposition of enzyme substrate (Zhao *et al.* 2013).

Increment of chitinase activity was observed with increment of dead fungal biomass up to 1.5 g, then it was gradually decreased (Fig. 5).

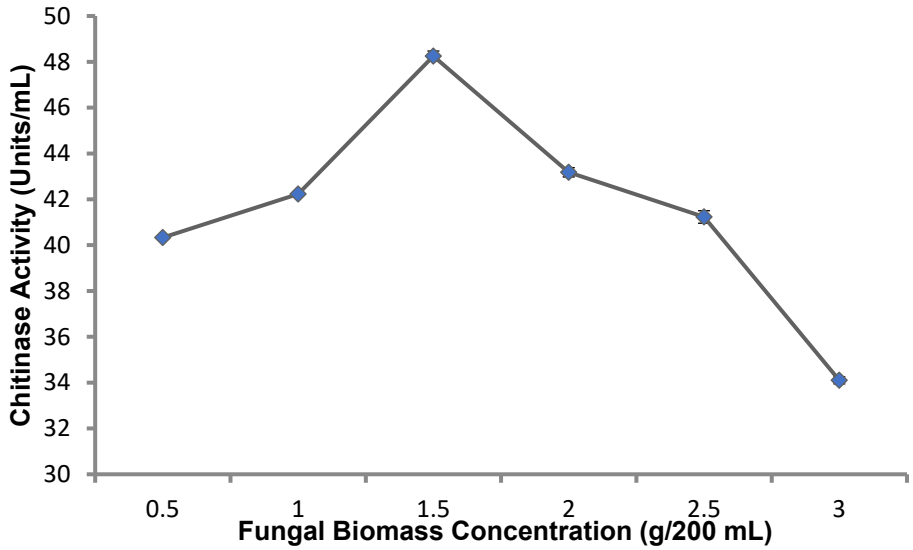

Fig. 5. Chitinase activity at different concentrations of dead fungal biomass. Error bars ± indicated standard deviation

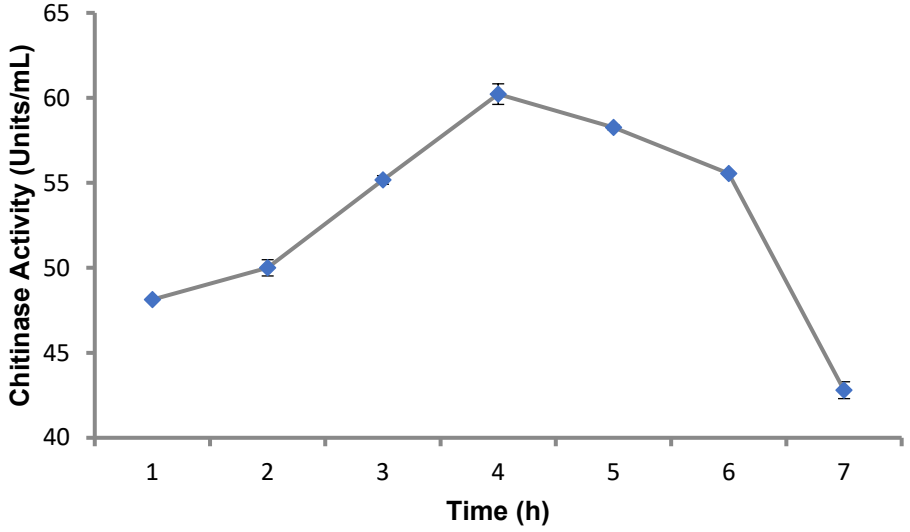

Fig. 6. Chitinase activity at different incubation times**.** Error bars ± indicated standard deviation

The inhibitory action of high substrate was probably due to the presence of some compounds in dead fungal biomass that interfere with chitinase activity. Chitinase activity was studied by Sridevi and Mallaiah (2008) at different concentrations 0.5 to 5% of chitin. They showed that 2 to 3% was the optimum concentration of enzyme activity, beyond which no increment of chitinase activity was observed with further increase of chitin concentration. Activity of chitinase was tested at different times. Maximum activity was observed at 4 h, after which further increase in time did not show any an increase in enzyme activity (Fig. 6). At the 1st h, the activity (48.1 Unit/mL) was more than the activity (42.8 Unit/mL) at 7 h.

Increasing chitinase activity was observed with increasing incubation temperature up to 50 °C. High temperature was more effective than low temperature for enzyme activity (Fig. 7). Increasing temperature may increase the enzyme activity *via* decreasing viscosity resulting from the substrate. The current findings were similar to results observed in another reports associated with chitinase activity.

The stability of *Pseudomonas aeruginosa* chitinase (Thompson *et al.* 2001) and *Penicillium oxalicum* chitinase (Rodríguez *et al.* 1995) was recorded at 50 °C and at less than 45 °C, respectively. As mentioned in a recent study (Emruzi *et al.* 2020), the optimum temperature for chitinase activity of *Serratia marcescens* was 50 °C. Also, the stability of enzyme was observed at 90 °C for 60 min.

Thermodynamic studies on chitinase activity at different temperatures ranging from 10 to 60 °C evaluated the behavior of the enzyme. As shown in Fig. 8, thermodynamic properties indicated that $\Delta H°$ and $\Delta S°$ were 126.1 (KJ mol^{-1}) and 431.5 (J mol^{-1} K^{-1}), respectively (Table 3). This means that this reaction is an endothermic reaction up to 60 °C with an increase in disorder after 60 °C. Variation in $\Delta G°$ values indicated that the reaction at 10 to 20 °C is a nonspontaneous reaction, and at 30 to 60 °C the reaction has a spontaneous nature.

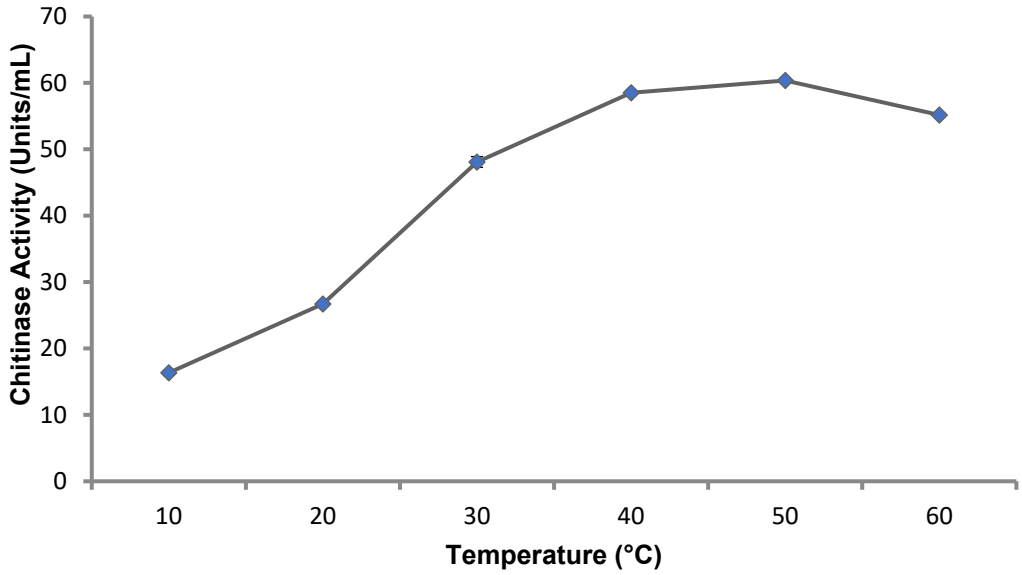

Fig. 7. Chitinase activity at different temperatures. Error bars ± indicated standard deviation

Table 3. Thermodynamic Parameters for Chitinase Activity at Different Temperatures

ΔH (KJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔG (KJ mol^{-1})					
		T = 10 °C	T = 20 °C	T = 30 °C	T = 40 °C	T = 50 °C	T = 60 °C
126.1	431.5	2.44	0.63	-3.3	-8.16	-15.1	-6.1

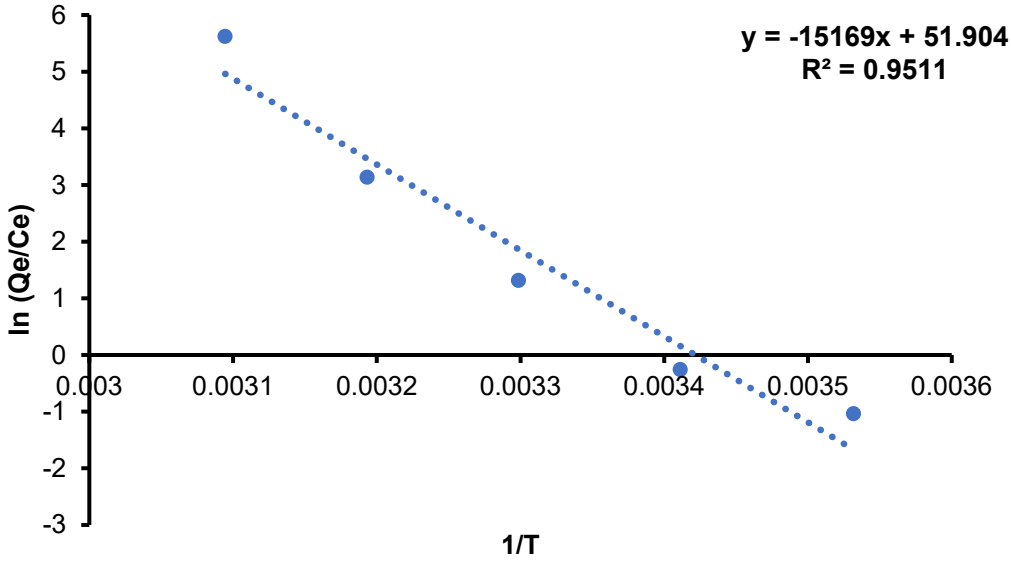

Fig. 8. Thermodynamic parameters of chitinase activity

The effect of different pH on chitinase productivity is visualized in Fig. 9. The optimum pH was 6, where the chitinase activity was 65.3 units/mL followed by pH 5. The obtained findings indicated that acidic pH was better than alkaline pH.

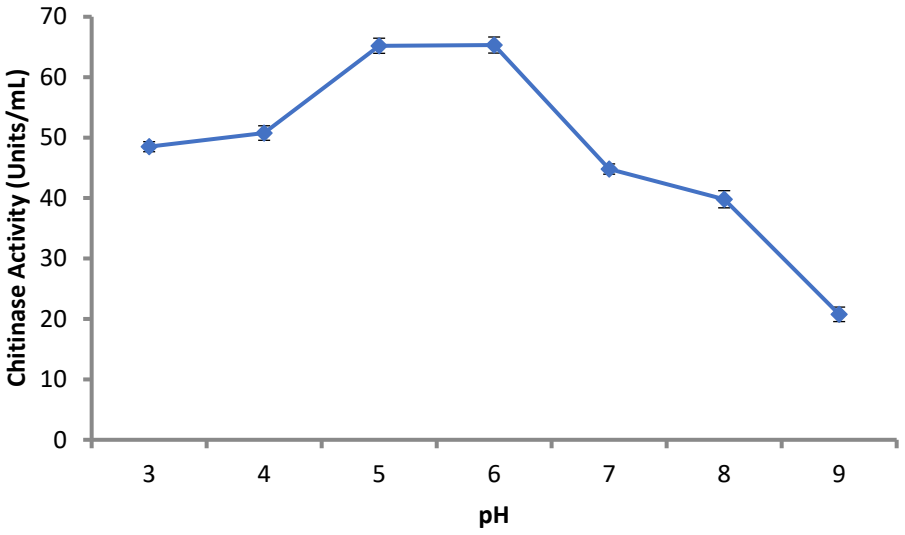

Fig. 9. Chitinase activity at different temperatures**.** Error bars ± indicated standard deviation

Activity of chitinase (48.5 units/mL) was more than its activity (20.8) at pH 9. The current results may be due to the fact that acidic conditions are more favorable for most fungi and their activities. A parallel behavior has been detected in other studies (Patel et al. 2019; Al-Rajhi *et al.* 2022a).

Antibacterial and antifungal activities of medium extract of fungus grown on medium with or without dead fungal biomass as inducer of antibiosis were documented (Table 4 and Fig. 10).

Table 4. Antimicrobial Activity of Culture Filtrate Extract of Fungus Growing With and Without Dead Fungal Biomass

Test Organism	Inhibition Zone (mm)					IF of A/C (%)	IF of B/C (%)
	A*	B*	C*	D*	E*		
P. aeruginosa	0.0 ± 0.00	2.3 ± 0.06	2.6 ± 0.12	1.8 ± 0.10	3.2 ± 0.12	100	23.07
E. coli	2.1 ± 0.06	2.2 ± 0.14	3.5 ± 0.06	2.2 ± 0.06	3.6 ± 0.21	4.54	2.700
B. subtilis	2.3 ± 0.06	2.8 ± 0.06	3.4 ± 0.17	2.3 ± 0.17	3.8 ± 0.17	0.00	10.52
A. fumigatus	0.0 ± 0.00	0.8 ± 0.02	0.8 ± 0.06	0.8 ± 0.04	1.2 ± 0.06	100	35.83
M. circinelloides	0.0 ± 0.00	0.7 ± 0.12	0.7 ± 0.06	0.0 ± 0.00	0.8 ± 0.06	-	13.58
C. albicans	0.0 ± 0.00	2.2 ± 0.21	2.8 ± 0.12	3.2 ± 0.18	3.4 ± 0.03	100	15.78

*Note: Without dead biomass (A); With dead biomass (B); Antibiotic/antifungal (C); Without dead biomass + Antibiotic/antifungal (D); With dead biomass + Antibiotic/antifungal (E). ±, indicated standard deviation.

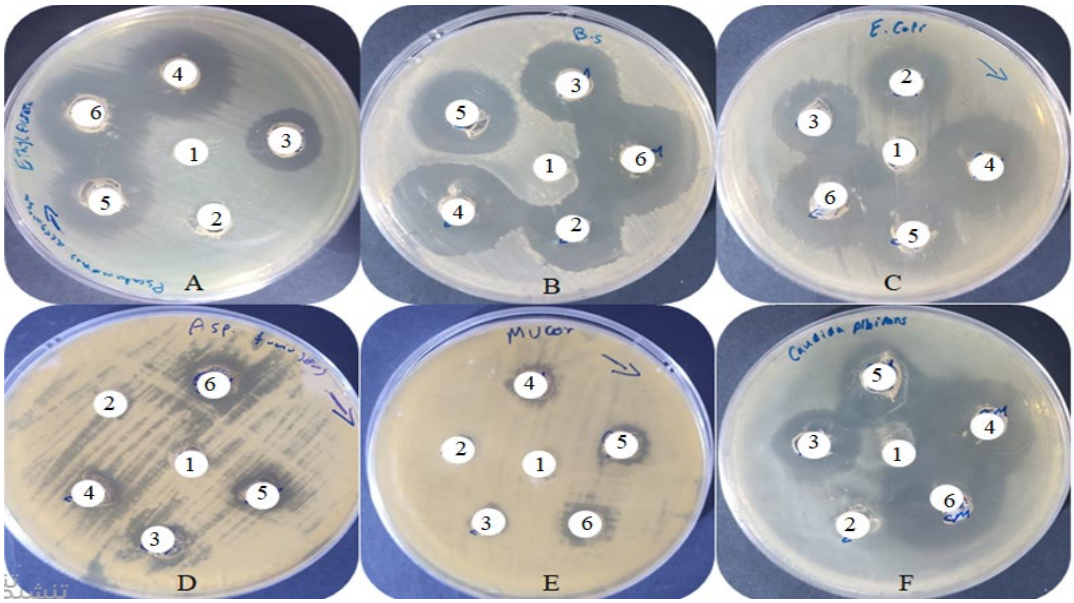

Fig. 10. Antibiosis of culture filtrate extract of fungus growing with and without dead fungal biomass against: *P. aeruginosa* (A), *B. subtilis* (B), *E. coli* (C), *A. fumigatus* (D), *M. circinelloides* (E), and *C. albicans* (F); Negative control extracted solvent (1), Without dead biomass (2), Antibiotic/antifungal (4), Without dead biomass + Antibiotic/antifungal (3), With dead biomass (5), With dead biomass + Antibiotic/antifungal (6)

The extract without dead biomass exhibited antibacterial activity against *E. coli* and *B. subtilis* but not against *P. aeruginosa*. Furthermore, the extract without dead biomass did not exhibit antifungal activities against tested fungi. In contrast, good antibacterial activity was recored using extracted medium ammended with dead biomass where the inhibition zone was 2.3, 2.2, 2.8, 0.8, 0.7, and 2.2 mm compared with the

antibiotic/antifungal. Moreover, the combination between antibiotic/antifungal and the extracted medium ammended with dead biomass exhibited synergistic action with increasing fold represented by 23.1, 2.7, 10.5, 35.8, 13.6, and 15.8% against *P. aeruginosa*, *E. coli, B. subtilis*, *A. fumigatus*, *M. circinelloides*, and *C. albicans*, respectively (Table 4). The observed increased activity was a result of a combination between the extract without dead biomass and antibiotic/antifungal due to the activity to antibiotic/antifungal but not to the extract. The differences among activities towards tested organisms may be associated with active contents of the extract and components of bacterial and fungal cell walls. The antimicrobial activity may be due to the fungal secondary metabolites, as mentioned in GC/MS analysis or to chitinolytic activity. According to Brzezinska and Jankiewicz (2012), *Fusarium culmorum*, *F. solani*, and *Rhizoctonia solani* growth was inhibited but *Botrytis cinerea*, *Alternaria alternata*, and *F. oxysporum* growth was not inhibited by Chitinase produced by *Aspergillus niger* (Brzezinska and Jankiewicz 2012).

CONCLUSIONS

1. Chitinase production and antibiosis with creation of different secondary metabolites by *A. fumigatus* were stimulated by the presence of fungal dead biomass as mycostimulator.
2. Optimum production of chitinase was observed using 1.5 g of dead fungal biomass and pH 6.
3. Additionally, the highest activity of chitinase was recorded at 4 h and 50 °C.
4. Thermodynamic properties of chitinase described that the reaction is endothermic up to 60 °C, after 60 °C the reaction became disordered.

ACKNOWLEDGMENTS

Authors are thankful to Princess Nourah bint Abdulrahman University Researchers (supporting project number PNURSP2022R217) and Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

REFERENCES CITED

Abdelghany, T. M. (2014). "Eco-friendly and safe role of *Juniperus procera* in controlling of fungal growth and secondary metabolites," *Journal of Plant Pathology and Microbiology* 5(3), 231. DOI:10.4172/2157-7471.1000231

Abdelghany, T. M., and Bakri, M. B. (2019). "Effectiveness of a biological agent (*Trichoderma harzianum* and its culture filtrate) and a fungicide (methyl benzimacold-2-ylcarbamate) on the tomato rotting activity (growth, celluloytic, and pectinolytic activities) of *Alternaria solani*," *BioResources* 14(1), 1591-1602. DOI: 10.15376/biores.14.1.1591-1602

Abdelghany, T. M., Bakri, M. M., Al-Rajhi, A. M. H., Al Abboud, M. A., Alawlaqi, M. M., and Shater, A. R. M. (2020). "Impact of copper and its nanoparticles on growth, ultrastructure, and laccase production of *Aspergillus niger* using corn cobs

wastes," *BioResources* 15(2), 3289-3306. DOI: 10.15376/biores.15.2.3289-3306

Abdelghany, T. M., Ganash, M., Bakri, M. M., and Al-Rajhi, A. M. H. (2018). "Molecular characterization of *Trichoderma asperellum* and lignocellulolytic activity on barley straw treated with silver nanoparticles," *BioResources* 13(1), 1729-1744. DOI: 10.15376/biores.13.1.1729-1744

Abdelghany, T. M., Yahya, R., Bakri, M. M., Ganash, M., Amin, B. H., and Qanash, H. (2021). "Effect of *Thevetia peruviana* seeds extract for microbial pathogens and cancer control," *International Journal of Pharmacology* 17(8), 643-655. DOI: 10.3923/ijp.2021.643.655

Al-Rajhi, A. M., Asmaa, A. A., Reham, Y., and Abdelghany, T. M. (2022a). "Induction of hydrolytic enzyme production and antibiosis via a culture of dual fungal species isolated from soil rich with the residues of woody plants in Saudi Arabia," *BioResources* 17(2), 2358-2371. DOI: 10.15376/biores.17.2.2358-2371

Al-Rajhi, A. M., Reham, Y., Mohamed, M. A., Mohamed, A. F., Basma, H. A., and Abdelghany, T. M. (2022b). "Copper oxide nanoparticles as fungistat to inhibit mycotoxins and hydrolytic enzyme production by *Fusarium incarnatum* isolated from garlic biomass," *BioResources* 17(2), 3042-3056. DOI: 10.15376/biores.17.2.3042-3056

Baldrian, P., Větrovský, T., Cajthaml, T., Dobiášová, P., Petranková, M., Šnajdr, J., and Eichlerová, I. (2013). "Estimation of fungal biomass in forest litter and soil," *Fungal Ecology* 6(1), 1-11. DOI: 10.1016/j.funeco.2012.10.002

Bharat, K., Barshana, B., Tanvi, K., Aaron, R., Monish, B., and Suniti, A. (2021). "Drug repurposing: *In silico* modeling of Mucor mycosis," *Journal of Scientific Research and Reports* 27(10), 53-61. DOI: 10.9734/JSRR/2021/v27i1030449

Bowman, S. M., and Free, S. J. (2006). "The structure and synthesis of the fungal cell wall," *BioEssays* 28(8), 799-808. DOI: 10.1002/bies.20441

Bradford, M. M. (1976). "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Analytical Biochemistry* 72(1-2), 248-254. DOI: 10.1016/0003-2697(76)90527-3

Brakhage, A. A., and Schroeckh, V. (2011). "Fungal secondary metabolites - strategies to activate silent gene clusters," *Fungal Genetics and Biology* 48(1), 15–22. DOI: 10.1016/j.fgb.2010.04.004

Brzezinska, M. S., and Jankiewicz, U. (2012). "Production of antifungal chitinase by *Aspergillus niger* lock 62 and its potential role in the biological control," *Current Microbiology* 65(6), 666-672. DOI: 10.1007/s00284-012-0208-2

Chang, W. T., Chen, Y. C., and Jao, C. L. (2007). "Antifungal activity and enhancement of plant growth by *Bacillus cereus* grown on shellfish chitin wastes," *Bioresource Technology* 98(6), 1224–1230. DOI: 10.1016/j.biortech.2006.05.005

Chatterjee, S., Kuang, Y., Splivallo, R., Chatterjee, P., and Karlovsky, P. (2016). "Interactions among filamentous fungi *Aspergillus niger*, *Fusarium verticillioides* and *Clonostachys rosea*: Fungal biomass, diversity of secreted metabolites and fumonisin production," *BMC Microbiology* 16, 1-13. DOI: 10.1186/s12866-016-0698-3

Collado, I. G., and Viaud, M. (2016). "Secondary metabolism in *Botrytis cinerea*: Combining genomic and metabolomic approaches," in: *Botrytis – the Fungus, the Pathogen and its Management in Agricultural Systems*, S. Fillinger, and Y. Elad (eds.), Springer, Cham, Switzerland, pp. 291-313. DOI: 10.1007/978-3-319-23371-0_15

da Silva, M. V., Colodel, E. M., da Costa, A. M., Driemeier, D. L., Santi, S. A., Staats, C. C., and Vainstein, M. H. (2005). "Cuticle-induced endo/exoacting chitinase CHIT30 from *Metarhizium anisopliae* is encoded by an ortholog of the *chi3* gene," *Research Microbiol*ogy 156(3), 382-392. DOI: 10.1016/j.resmic.2004.10.013

Deng, S., Harman, G. E., Lorito, M., and Penttilä, M. (2007). "Overexpression of an endochitinase gene (*ThEn-42*) in *Trichoderma atroviride* for increased production of antifungal enzymes and enhanced antagonist action against pathogenic fungi," *Applied Biochemistry and Biotechnology* 142(1), 81-94. DOI: 10.1007/s12010-007-0012-9

Domsch, K. H., Gams, W., and Anderson, T. (1980). *Compendium of Soil Fungi*, Academic Press (London) Ltd., London, UK.

Dukare, A., Paul, S., and Arambam, A. (2020). "Isolation and efficacy of native chitinolytic rhizobacteria for biocontrol activities against *Fusarium* wilt and plant growth promotion in pigeon pea (*Cajanus cajan* L.)," *Egyptian Journal Biological Pest Control* 30, article no. 56. DOI: 10.1186/s41938-020-00256-7

Duraipandiyan, V., Al-Harbi, N. A., Ignacimuthu, S., and Muthukumar, C. (2012). "Antimicrobial activity of sesquiterpene lactones isolated from traditional medicinal plant, *Costus speciosus* (Koen ex. Retz.) Sm.," *BMC Complementary and Alternative Medicine* 12, article no. 13. DOI: 10.1186/1472-6882-12-13

Ekundayo, F.O., Folorunsho, A.E., Ibisanmi, T.A., and Olabanji, O. B. (2022). "Antifungal activity of chitinase produced by *Streptomyces* species isolated from grassland soils in Futa Area, Akure," *Bull. Natl. Res. Cent.* 46, 95. DOI: 10.1186/s42269-022-00782-4

Elieh-Ali-Komi, D., and Hamblin, M. R. (2016). "Chitin and chitosan: Production and application of versatile biomedical nanomaterials," *International Journal of Advanced Research* 4(3), 411–427.

El-Tarabily, K. A., Soliman, M. H., Nassar, A. H., Al-Hassani, H. A., Sivasithamparam, K., McKenna, F., and Hardy, G. E. S. J. (2000). "Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes," *Plant Pathololog*y 49, 573-583. DOI: 10.1046/j.1365-3059.2000.00494.x

Emruzi, Z., Keshavarz, M., Gholami, D., Aminzadeh, S., and Noori, A. (2020). "Kinetic and thermo-inactivation thermodynamic parameters of a novel isolated *Serratia marcescens* B4A chitinase," *Biomacromolecular Journal* 6(1), 46-55.

Fischer, N. H., Lu, T. Cantrell, C. L., Castañeda-Acosta, J., Quijano, L., and Franzblau, S. G. (1998). "Antimycobacterial evaluation of germacranolides in honour of professor GH Neil Towers 75th birthday," *Phytochemistry* 49(2), 559-564. DOI: 10.1016/S0031-9422(98)00253-2

Gilvanova, E. A., Aktuganov, G. E., Safina, V. R., Milman, P. Y., Lopatin, S. A., Melintiev, A. I., Galimzianova, N. F., Kuzmina, L. Y., and Boyko, T. F. (2022). "Characterization of thermotolerant chitinase from the strain *Cohnella* sp. IB P-192 and its application for the production of bioactive chitosan oligomers," *Appl. Biochem. Microbiol.* 58**,** 143-154. DOI: 10.1134/S0003683822020077

Gupta, R., Saxena, R. K., Chaturvedi, P., and Virdi J. S. (1995). "Chitinase production by *Streptomyces viridificans*: Its potential in fungal cell wall lysis," *Journal of Applied Bacteriology* 78(4), 378-83. DOI: 10.1111/j.1365-2672.1995.tb03421.x

Karam, E. A., Wahab, W. A. A., Saleh, S. A. A., Hassan, M. E., Kansoh, A. L., and Esawy, M. A. (2017). "Production, immobilization and thermodynamic studies of free and immobilized *Aspergillus awamori* amylase," *International Journal of*

Biological Macromolecules 102, 694-703. DOI: 10.1016/j.ijbiomac.2017.04.033

Luna-Herrera, J., Costa, M., Gonzalez, H., Rodrigues, A., and Castilho, P. (2007). "Synergistic antimycobacterial activities of sesquiterpene lactones from *Laurus* spp.," *Journal of Antimicrobial Chemotherapy* 59(3), 548-552. DOI: 10.1093/jac/dkl523

Mohanad, J. K., Ghaidaa, J. M., and Haider, M. H. (2016). "Analysis of bioactive metabolites from *Candida albicans* using (GC-MS) and evaluation of antibacterial activity," *International Journal of Pharmaceutical and Clinical Research* 8(7), 655-670.

Mostafa, F. A., Abdel Wahab, W. A., Salah, H. A., Nawwar, G. M., and Esawy, M. A. (2018). "Kinetic and thermodynamic characteristic of *Aspergillus awamori* EM66 levansucrase," *International Journal of Biological Macromolecules* 119, 232-239. DOI: 10.1016/j.ijbiomac.2018.07.111

Nofal, A. M., El-Rahman, M. A., Alharbi, A. A., and Abdelghany, T. M. (2021a). "Ecofriendly method for suppressing damping-off disease caused by *Rhizoctonia solani* using compost tea," *BioResources* 16(3), 6378-6391. DOI: 10.15376/biores.16.3.6378-6391

Nofal, A. M., Abdelghany, T. M., and Abd-EL-Hamed, W. F. (2021b). "Significance of local *Trichoderma* isolates in controlling *Pythium ultimum* and *Rhizoctonia solani* on bean in Egypt," *Egyptian Journal of Phytopathology* 49(2), 131-140. DOI: 10.21608/EJP.2021.110177.1051

Nofal, A. M., El-Rahman, M. A., Abdelghany, T. M., and Mahmoud, A. E. (2021c). "Mycoparasitic nature of Egyptian *Trichoderma* isolates and their impact on suppression *Fusarium* wilt of tomato," *Egyptian Journal of Biological Pest Control* 31, Article Number 103. DOI: 10.1186/s41938-021-00450-1

Patel, N., Shahane, S., Majumdar, R., and Mishra, U. (2019). "Mode of action, properties, production, and application of laccase: A review," *Recent Pat. Biotechnol.* 13(1), 19-32. DOI: 10.2174/1872208312666180821161015.

Prado-Cabrero, A., Scherzinger, D., Avalos, J., and Al-Babili, S. (2007). "Retinal biosynthesis in fungi: Characterization of the carotenoid oxygenase CarX from *Fusarium fujikuroi*," *Eukaryotic Cell* 6(4), 650-657. DOI: 10.1128/EC.00392-06

Rana, K. I., and Farhana, N. A. (2019). "Chitinases potential as bio-control," *Biomedical Journal of Scientific & Technical Research* 14(5), 10994-11001. DOI: 10.26717/BJSTR.2019.14.002629

Raper, K. B., and Fennell, D. I. (1973). *The Genus Aspergillus*, Krieger Publishing Company, Huntington, New York, NY, USA.

Rateb, M. E., Hallyburton, I., and Houssen, W. E. (2013). "Induction of diverse secondary metabolites in *Aspergillus fumigatus* by microbial co-culture," *RSC Advances* 3(34), 14444-14450. DOI: 10.1039/c3ra42378f

Renu, P., and Dinesh, K. (2015). "Study of chemical composition in wild edible mushroom *Pleurotus cornucopiae* (Paulet) from Himachal Pradesh, India by using Fourier transforms infrared spectrometry (FTIR), gas chromatography-mass spectrometry (GC-MS) and X-ray fluorescence (XRF)," *Biological Forum-An International Journal* 7(2), 1057-1066.

Rodríguez, J., Copa-Patiño, J. L., and Pérez-Leblic, M. I. (1995). "Purification and properties of a chitinase from *Penicillium oxalicum* autolysates," *Letters in Applied Microbiology* 20(1), 46-49. DOI: 10.1111/j.1472-765x.1995.tb00404.x

Schumacher, J., de Larrinoa, I. F., and Tudzynski, B. (2008). "Calcineurin-responsive zinc finger transcription factor CRZ1 of *Botrytis cinerea* is required for growth,

development, and full virulence on bean plants," *Eukaryot Cell* 7(4), 584-601. DOI: 10.1128/EC.00426-07

Shaikh, S. S., and Sayyed, R. Z. (2015). "Role of plant growth promoting rhizobacteria and their formulation in biocontrol of plant diseases," in: *Plant Microbes Symbiosis: Applied Facets*, N. K. Arora (ed.), Springer, New Delhi, India, pp. 337-351.

Qanash, H., Yahya, R., Bakri, M. M., Bazaid, A. S., Qanash, S., Shater, A. F., and Abdelghany, T. M. (2022). "Anticancer, antioxidant, antiviral and antimicrobial activities of Kei Apple (*Dovyalis caffra*) fruit," *Scientific Reports* 12, article no. 5914. DOI: 10.1038/s41598-022-09993-1

Sridevi, M., and Mallaiah, K. V. (2008). "Factors effecting chitinase activity of *Rhizobium* sp. from *Sesbania sesban*," *Biologia* 63(3), 307-312. DOI: 10.2478/s11756-008-0070-7

Starke, R., Morais, D., Větrovský, T., Mondéjar, R. L., Baldrian, P., and Brabcová, V. (2020). "Feeding on fungi: Genomic and proteomic analysis of the enzymatic machinery of bacteria decomposing fungal biomass," *Environmental Microbiology* 22(11), 4604-4619. DOI: 10.1111/1462-2920.15183

Thompson, S. E., Smith, M., Wilkinson, M. C., and Peek, K. (2001). "Identification and characterization of a chitinase antigen from *Pseudomonas aeruginosa* strain 385," *Applied Environmental Microbiology* 67(9), 4001-4008. DOI: 10.1128/AEM.67.9.4001-4008.2001

Tronsmo, A., and Harman, G. E. (1993). "Detection and quantification of N-acetyl-p-D-glucosaminidase, chitobiase and endochitinase in solutions and on gels," *Analytical Biochemistry* 208(1), 74-79. DOI: 10.1006/abio.1993.1010

Ueno, H., Miyashita, K., Swada, Y., and Oba, Y. (1990). "Purification and some properties of extracellular chitinase from *Streptomyces* sp. S-84," *The Journal of General and Applied Microbiology* 36(6), 377-392. DOI: 10.2323/jgam.36.377

Vinothkanna, A., Manivannan, P., Muralitharan, G., and Sekar, S. (2014). "*In silico* probing of anti-arthritic potential of traditionally fermented ayurvedic polyherbal product balarishta reveals lupeol and desulphosinigrin as efficient interacting components with urec," *International Journal of Pharmacy and Pharmaceutical Sciences* 6(10), 469-475.

Vyas, P., and Deshpande, M. V. (1989). "Chitinase production by *Myrothecium verrucaria* and its significance for fungal mycelia degradation," *Journal of General and Applied Microbiology* 35(5), 343-350. DOI: 10.2323/jgam.35.343

Webster, J., and Weber, R. S. (2007). *Introduction to Fungi*, Cambridge University Press, Cambridge.

Zeglin, L. H., and Myrold, D. D. (2013). "Fate of decomposed fungal cell wall material in organic horizons of old-growth Douglas-fir forest soils," *Soil Science Society of America Journal* 77(2), 489-500. DOI: 10.2136/sssaj2012.0204

Zhao, J., Xue, Q. H., Niu, G. G., Xue, L., Shen, G. H., and Du, J. Z. (2013). "Extracellular enzyme production and fungal mycelia degradation of antagonistic *Streptomyces* induced by fungal mycelia preparation of cucurbit plant pathogens," *Annals of Microbiology* 63, 809-812. DOI: 10.1007/s13213-012-0507-7

Article submitted: March 25, 2022; Peer review completed: May 28, 2022; Revised version received and accepted: May 29, 2022; Published: June 1, 2022.
DOI: 10.15376/biores.17.3.4378-4394